Special Techniques, C: Sex Determination

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Cranes are considered **monomorphic**. Although males average slightly larger, size is not always reliable as an indicator of sex. Subadult and adult cranes can normally be sexed by vocalizations, and there is potential for sexing chicks by sonagram analysis (Carlson 1991). General behavior can also indicate, but not diagnose, sex. **Fecal steroid analysis** can be used to sex nearly all subadults, adults, and many young birds, and cranes of all ages can be **genetically sexed** by DNA probe, by microscopic examination of their chromosomes (karyotyping), or by **total DNA** measurements. Cranes that are 3 months old or older can be gonadally sexed via **laparoscopy**.

Vocalizations

Cranes approach "vocal maturity" when they are 8 to 12 months old (Walkinshaw 1973; Nesbitt 1975; personal observation). Vocally mature cranes have sex-specific differences in their **Unison-calls** and, in many species, their **Guard-calls** (Archibald 1976a, 1976b; Carlson and Trost 1992). Sonagram analysis of vocalizations is an accurate way to sex adult and subadult cranes (Archibald 1976a, 1976b; Carlson 1991; Carlson and Trost 1992), and perhaps most Whooping Crane chicks (G. Carlson, Idaho State University, Pocatello, Idaho, personal communication).

Guard-call

Cranes Guard-call by giving one loud burst, pausing one or more seconds, giving another call, and so on. Mates often give synchronous Guard-calls. Male cranes have lower pitched voices than females in all species except the African Crowned Cranes. These differences are most obvious when cranes of both sexes are calling, but experienced persons can sex birds without reference to another crane.

Unison-call

Archibald (1976a, 1976b) studied the Unison-call display in detail and found ways to differentiate the sexes of all but the African Crowned Cranes. In most species, sexes have different **stances** (Fig. 11C.1), and/or different **number of notes** given, during the Unison-call. The principal differences in Unison-calls that indicate sex fall into five groups: (1) in some species, the male raises his elbow and/or lowers his primaries during the display, while the female does not; (2) in some species, the female usually, or always, begins the display; (3) in some species, the female gives two to three notes for each male note of the display; (4) in some species, the male holds his bill more vertically, or further over his back beyond the vertical, than the female; and (5) in most species, the female's voice is higher pitched than the male's.

Black Crowned and Gray Crowned Cranes. Crowned Cranes usually incorporate a series of **ka-wonk** like Guard-calls into their Unison-calls. The slight differences in pitch between the sexes are not sufficient to diagnose a crane's sex by ear. The Black Crowned Crane usually uses Guard-calls exclusively. The only sexual difference in this species is the female's higher pitched ka-wonk calls. Gray Crowned Cranes, on the other hand, use low-pitched **booms** as their Unison-calls, and employ ka-wonks as their Guard-calls. The female's ka-wonk calls average slightly longer than the male's in this species. Although both sexes have similarly pitched ka-wonk calls, the female's booming calls are lower pitched than the male's.

Demoiselle Crane. The female usually begins the Unison-call by throwing her head back beyond the vertical, while the male follows her first note by giving lower pitched, longer, and more broken notes. He holds his neck vertical with the bill elevated 45° above the horizontal. The female either holds her initial position or gradually returns her head to a horizontal position. The female's voice is higher pitched than the male's.

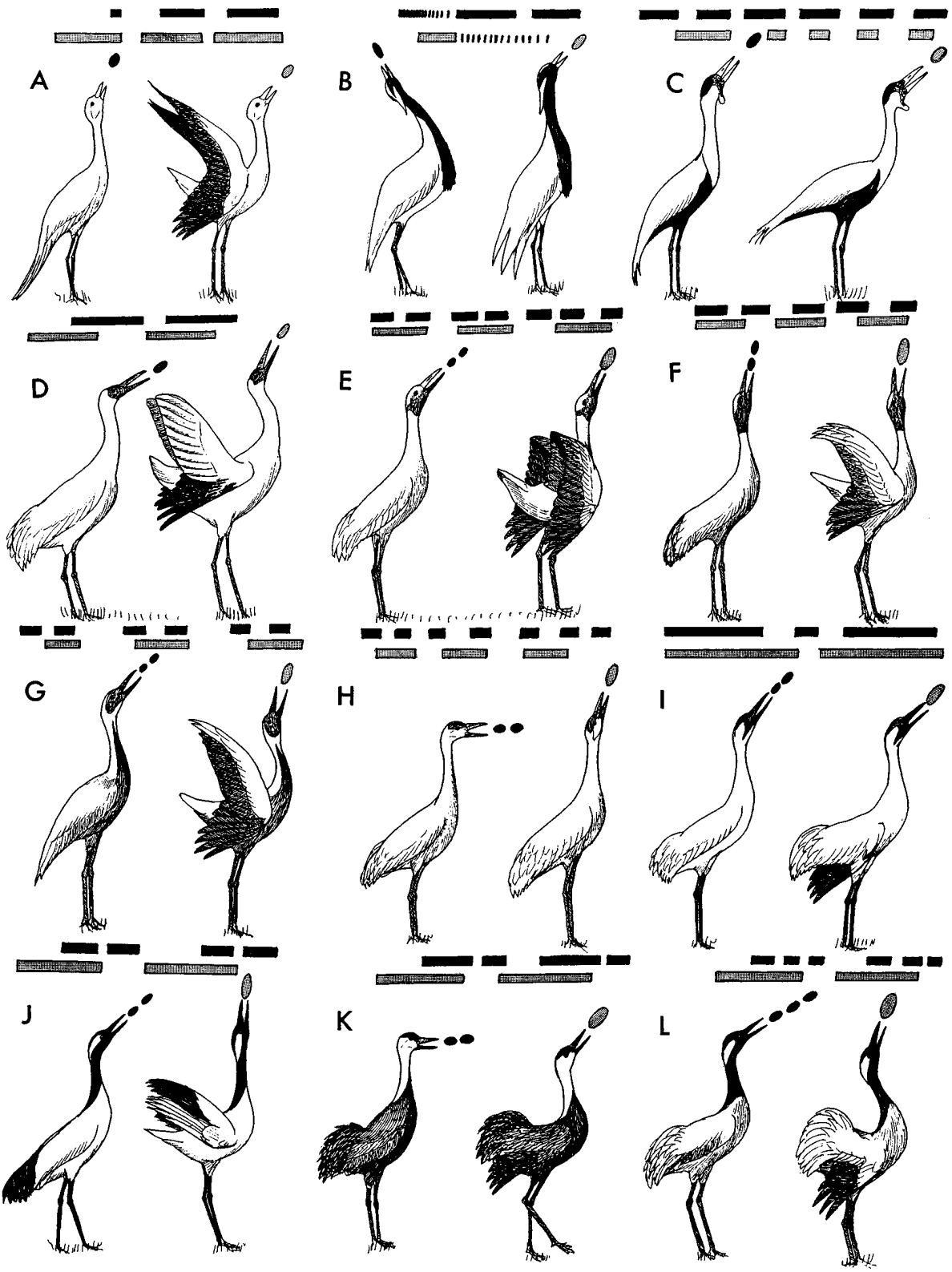


FIG. 11C.1. *Male and female Unison-call postures for the following crane species: (A) Blue, (B) Demoiselle, (C) Wattled, (D) Siberian, (E) Brolga, (F) Sarus, (G) White-naped, (H) Sandhill, (I) Whooping, (J) Red-crowned, (K) Hooded, and (L) Eurasian. Durations of vocal - izations are indicated by shaded bars (male) and black bars (female). "Balloons" indicate typical number of female calls per male call. Based on Archibald (1976b).*

ART PAUL A. JOHNSGARD. USED BY PERMISSION FROM JOHNSGARD (1983).

Blue Crane. The female usually throws her neck back 20° beyond the vertical at the start of the call. The male throws his head and neck back even further, about 40° beyond the vertical. The male raises his humeri above the back during the display, while the female does not.

Wattled Crane. The female begins the call by rapidly lowering her head to shoulder level, then rapidly raising her neck with the head about 30° in front of the vertical. She maintains this posture throughout the call. The male quickly follows the female's introduction with a long and partly broken call, then gives longer, fewer, and lower pitched notes than the female as in the Indian Sarus Crane. With his last note, which is long and broken like the first, he raises his humeri 20° above the back. The female does not elevate her wings.

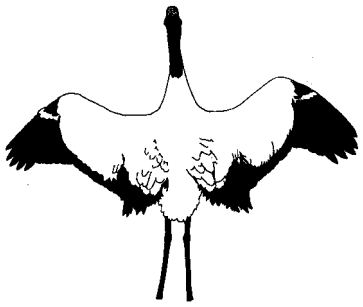
Siberian Crane. The female's voice is higher pitched than the male's, but because some males are higher pitched than others, this male-female difference is sometimes apparent only when the two sexes can be compared. Either sex may lower its primaries or walk during the display. One sex-specific difference is that when the male begins the call, he swings his head up quickly and then throws it down near his chest with a long preliminary note. Sometimes, however, the female begins the call.

Other Grus Cranes. In most of the remaining **Grus** cranes the female gives two to three notes for each male note of the Unison-call. There are sexual differences in the stance during the display in all of these species.

In the **Sandhill Crane**, the female usually initiates the call with an explosion of rapid notes. The male sometimes begins the call. The female gives two or three notes for every male note. The male holds his head further back and with the bill more vertical than the female. The female holds her bill nearly level, but quickly flips her bill up with each note. The female's voice is higher pitched than the male's in both the Unison- and Guard-calls. The female's Guard-call notes are more broken than the male's.

In the **Sarus**, **Brolga**, and **White-naped Cranes**, the male raises his humeri high above the back and completely lowers his primaries during the Unison-call, creating an impressive visual display (Fig. 6.10). The female does not move her wings during the display, and her voice is higher pitched. The female usually begins the Unison-call in two of these species, and always does so in the White-naped Crane.

In the **Common**, **Hooded**, **Whooping**, **Black-necked**, and **Red-crowned Cranes**, the male usually raises his humeri during the display, often raising and lowering them with each note. The female does not raise her humeri, except occasionally in the Whooping Crane and rarely in the Common Crane. In these two species, the male raises his humeri more often than the female and extends his head far over his body, while the female has a more vertical stance. In each of these five species the female gives two or three notes for each male note of the Unison-call, and her voice is higher pitched.



General Differences in Behavior Between the Sexes

In wild cranes, the male nearly always leads the female when a pair moves from place to place (Tacha 1987). The male is the principal defender of the pair and tends to adopt more erect and aggressive postures with his head held higher than the female's. The male often spends more time watching for intruders than the female. The female more often shows neck-retracted submissive postures when an intruder approaches. By contrast, the female in captivity very often, and perhaps most of the time, initiates dance and calling activities.

Captive, hand-reared males often attack people and may become more aggressive after being paired. Hand-reared females may also approach people but are usually more submissive than males. Once paired, the female may become aggressive like the male. The male of a pair usually displays more intensely, and approaches an intruder closer, than the female.

Bare-skin- or **Wattle-expansion** reflects increased aggression in cranes. Males usually expand their crowns or wattles more than females. In the Wattled Crane, an aggressive male may have larger wattles than his mate during conflicts.

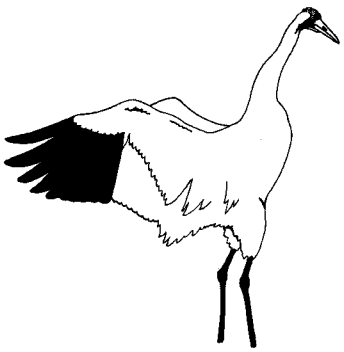
Size

Male cranes are usually heavier and taller than females, but there is considerable overlap between the sexes (Walkinshaw 1973; Johnsgard 1983). It is possible to sex larger-than-average males and smaller-than-average females by this method. Captive males of six species averaged 14.5-28.5% heavier, had 2.5-11.7% longer culmens, and 3.3-11.1% longer tarsi than conspecific females (Swengel 1992). Because cranes are usually heavier in captivity, their weights cannot be compared with wild birds.

There is also considerable **seasonal variation in weight**. Winter weights may be greater than summer body weights by 35% or more. For temperate and arctic-nesting cranes, weight is much more variable, hence much less useful as an indicator of sex (Song 1991; Swengel 1992).

Multi-parameter discriminant functions are now available to sex adults of two species. Murata et al. (1988) found that a discriminant function combining tail, wing chord, tarsus, and culmen measurements could safely sex captive Red-crowned Cranes. Markin and Krever (1992) developed a similar function using culmen, tarsus, and middle toe to sex wild Common Cranes. They have also created discriminant functions for sexing Demoiselle, Siberian, Sandhill, White-naped, and Red-crowned Cranes (V. Krever, Central Laboratory of Game Management, Moscow, Russia, unpublished data [on file at ICF]). Because of feather wear, wing chord and tail measurements are not very useful for sexing cranes.

In 20 wild Brolga pairs, Blackman (1971) found that males were always heavier and had longer heads, tarsi, and bodies than their mates. This suggests that female Brolgas, and possibly other cranes, choose mates larger than themselves.



Genetic Sexing

Karyotyping

Male cranes have two Z sex chromosomes, while females have one Z and one W chromosome (Fig. 11C.2). A crane's sex can be determined by the number of large chromosomes (**macrochromosomes**) (Rasch and Kurtin 1976; Sasaki and Takagi 1981; Goodpasture et al. 1992); cranes have four to five pairs of macrochromosomes besides the sex chromosomes. The Z chromosome is about as large as the fourth or fifth largest pair of chromosomes, while the W chromosome is much smaller. In the female, dividing mitotic cells in metaphase (chromosome spread) contain four or five paired and one unpaired macrochromosome.

Chromosomes can be obtained either from a blood sample or from the pulp of a growing feather. Some investigators karyotype dividing white blood cells (Takagi et al. 1972; Biedermanand Lin 1982) or feather pulp cells (Sasaki et al. 1968; Goodpasture et al. 1992). In birds, the feather pulp culture method is usually more successful in creating good chromosome spreads.

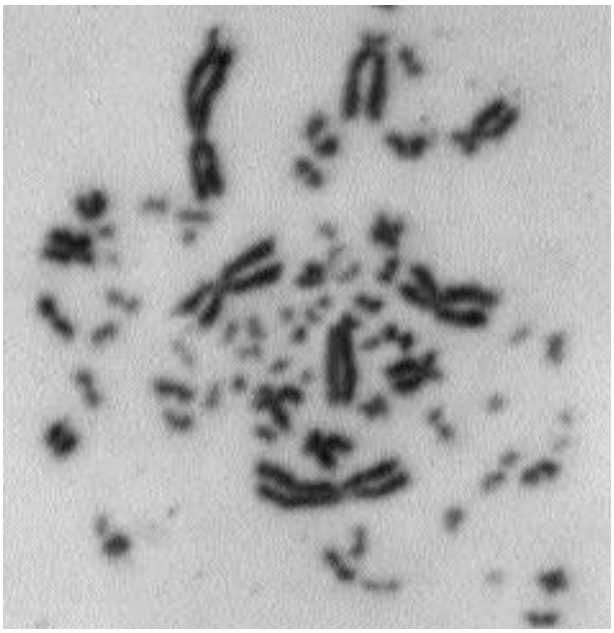


Fig. 11C.2. *Chromosome spread of a female Whooping Crane, with the Z chromosome identified. The W chromosome is one of the very small chromosomes located through a process of elimination. Males have two W chromosomes.*

Photo George F. Gee

Feather Pulp Sexing. For this method, a **large emerging feather** is pulled from the wing or tail. The feather can be obtained from a chick or an actively molting crane. If the bird to be sexed has no growing feathers, one or two large feathers can be pulled three to four weeks prior to the sampling date to stimulate regrowth. We pull two feathers to be sure at least one is growing (in the event one does not immediately grow back).

Gee (1982) found that pulling fully grown primaries from immature Sandhill Cranes often resulted in replacements that were deformed, were incapable of supporting flight, and were replaced several times in a year. To avoid this problem, we recommend using tail feathers or tertiaries. Pulling growing feathers seems to cause less harm to the feather follicle than pulling a fully grown feather.

When pulling growing feathers, be certain to remove the entire quill from the feather follicle. A large pair of pliers or hemostats provides a good purchase. Attach them as close to the base of the feather as possible, and always pull straight out. If a portion of the broken shaft is left in the follicle, bleeding may be prolonged. Although the condition is very rare, cranes have died from excessive blood loss through a broken blood quill of a large feather (G. F. Gee, Patuxent, personal communication). Most replacement secondaries are morphologically normal (personal observation), although their future loss and replacement rates have not been studied.

Pull the growing feather, wipe the shaft with alcohol, and cut it 2-3 cm from its insertion with sterile scissors. Immediately place the basal part of the feather in media provided by a cytological sexing lab. Contact the lab in advance to obtain shipping media and instructions for shipment. Samples should be shipped as soon as possible and should be refrigerated until shipping. Commercial feather pulp sexing labs in the United States include Avian Genetics Sexing Lab and Avigen (see Appendix).

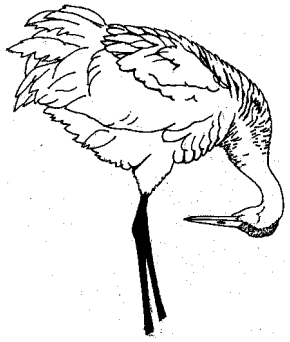
Blood Culture. Remove the crane's food five hours before drawing blood. Place 0.2 mL of sodium heparin (10,000 units/mL) into a sterile 3-cc syringe and work the plunger to coat the barrel of the syringe. Draw 2-3 cc of blood from the jugular or brachial vein, and invert the syringe several times to mix the blood with the heparin. Keep the blood refrigerated, and send it to a lab as soon as possible. In the United States and Canada, some research labs at major universities and zoos karyotype birds using blood cells (Takagi and Sasaki 1974; Biederman et al. 1982; Kumamoto 1984; see Appendix).

DNA Probe

Zoogen, Inc. (see Appendix) has developed a commercially available DNA probe technique (RFLP, Restriction Fragment Length Polymorphism) to differentiate male and female chromosomes. A 0.02-mL sample of a bird's blood (Halverson 1990), mixed with 70% ethanol, is sent by regular mail. This method has correctly sexed over 100 cranes at ICF and can be used on chicks.

Genome Size

A rarely used cytological sexing method is a measurement of the **total DNA content** of crane erythrocytes. Because the Z chromosome is larger than the W, males have larger genomes than females. Rasch (1976, 1977) found that males had 4-6% larger DNA Feulgen staining levels than females in their erythrocyte nuclei as determined by cytophotometry. This method requires only a few drops of blood.



Surgical Sexing

This method uses a laparoscope or otoscope (fiberoptic or otherwise) to view the sex organs through a small incision in the crane's left side (McDonald 1982; see description in Chapter 8). Cranes should be **anesthetized** before surgery to decrease both stress and the risk of accidental injury to vital organs if the bird struggles during laparoscopy. Because of the risk of injury, a veterinarian or trained technician should perform this operation. For birds in general, about 1 bird in 250 dies during laparoscopy (McDonald 1986).

On inspection, the **testes** are white to tan, rather cylindrical, and smooth on the surface. In young

cranes, they are small (0.1-0.2 mm by 0.5-1.21 mm) and usually avascular, while in mature cranes they have vascularized surfaces, are much larger (2-3 cm by 3-5 cm), and vary in size seasonally. The **ovary** is often not found in laparoscopy of young females. When visible, it may be flat, is pink to tan, and looks like "pebbled" fat. In subadult females, the ovary acquires a fine granular surface, and when the bird is mature, the follicles appear like a cluster of grapes. Nearly all female birds have only one functioning ovary (the left).

Vent Sexing

Most adult cranes, and some subadults or even yearlings, can be vent-sexed by methods described by Blackman (1971) and Tacha and Lewis (1979). While one person uses the massage technique to relax the bird as in artificial insemination (see Chapter 11A), a second person examines the cloaca, using the fingers to get a better view of critical features. Male cranes have two raised papillae side by side in the middle of the unmanipulated cloaca; these average 1 mm across and are usually lighter in color than the surrounding tissue. The presence of cloacal papillae in cranes older than one year is indicative of a male; their absence indicates a female. Most females greater than a year old have one or more corroborative cloacal features: an oviduct opening on the lower left of the cloaca when viewed from behind, and a small spot near the top of the oviduct, the bursa of Fabricius. Both are easiest to see during the breeding season and in reproductive females. Blackman (1971) illustrates these features, but the photos are upside down with respect to the view described above.

Fecal Steroid Sexing

The feces of female birds have higher immunoreactive estrogen/testosterone (E/T) ratios than males (Czekala and Lasley 1977). One fresh fecal sample is enough to determine sex. This method is about 90% accurate (Stavy et al. 1979), but seasonal and age variation can cause occasional overlap in the hormone ratios of the two sexes, especially during the nonbreeding season (Bercovitz 1983). Stavy et al. (1979) found that low testosterone values in adult birds was indicative of a female, regardless of the E/T ratio. Fecal samples should be analyzed immediately or frozen and stored at temperatures below –40° C.

Adult birds during the breeding season give the best results. However, waste material left in the egg when a bird hatches has been used for sexing hatchlings. The technique works even for tiny chicks because sex hormones are important in the sexual differentiation of bird embryos (T. Gross, Biotechnologies for the Ecological, Evolutionary, and Conservation Sciences, Gainesville, Florida, and A. Bercovitz, San Diego Zoo, San Diego, California, personal communications) and egg wastes sample a relatively large time period.

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